

**MOLECULAR EVALUATION OF SOME SALINITY
TOLERANT GENOTYPES OF SORGHUM
USING RT-PCR**

By

NOURHAN AIMAN HANAFI ABDEL-AZEEM

B.Sc. Agri. Sci., (Biotechnology), Fac. Agri., Ain Shams University, 2013

A Thesis Submitted in Partial Fulfillment

Of

The Requirements for the Degree of

MASTER OF SCIENCE

in

Agricultural Sciences

(Biotechnology in Arid Land)

Arid Land Agricultural Graduate Studies and Research Institute

Faculty of Agriculture

Ain Shams University

2020

ABSTRACT

Nourhan Aiman Hanafi Abdel-Azeem: Molecular Evaluation of Some Salinity Tolerant Genotypes of Sorghum Using RT-PCR, Unpublished Master thesis, Department of Biotechnology, Arid Land Agriculture graduate studies and Research Institute, Faculty of Agriculture, Ain Shams University, 2020.

Twenty sorghum (*Sorghum bicolor* L. {Monech}) genotypes were assessed for fingerprint using 10 ISSR primers. All tested ISSR primers yielded amplified products and generated 152 alleles (average of 15.2 bands/primer). Out of the 10 used primers, eight primers obtained 24 unique cultivar markers. However, molecular characterization revealed polymorphism percentage of a 100% for ISSR markers, indicating a high level of a polymorphism among the studied sorghum genotypes. On the other hand, cluster analysis using UPGMA analysis classified the 20 sorghum genotypes into two main groups. The cluster analysis showed a high genetic variation among the studied sorghum genotypes and the diversity of these genotypes was in an agreement with their source pedigree. The results of principle coordinate analysis (PCoA) were closely in line with those of the cluster analysis.

Eight salt tolerance-related traits were recorded after 30 days of salt stress treatment (10,000 ppm) such as fresh and dry weights, fresh and dry plant height, % germination, lux, numbers of leaves and roots traits, which performed from the previously distinguished genotypes such as A2 and B5 tolerant genotypes as well as B8 and A9 sensitive genotypes. Averages of salt tolerance related traits values were statically analyzed using ANOVA and LSD test. ANOVA one way test showed that there were significant differences among genotypes averages, whereas P value was less than 0.05 ($P < \alpha 0.05$). Therefore, the alternative hypothesis was accepted and the null hypothesis was rejected, as $H_a > H_0$ and $H_0: \mu_1 = \mu_2 = \mu_3 \dots = \mu_k$, which means that salt is negatively affected on salt tolerance-related traits in the studied genotypes. The results of

LSD test showed that A2 was the most tolerant genotype; while B8 was the most salt sensitive one as well as B5 and A9 were moderate in salt tolerance and sensitivity, respectively.

Moreover, RT-PCR was used to evaluate the gene expression of *SbBADH1* & *SbBADH2* salt tolerance related genes at various times of salt treatment. The expression of *SbBADH1* gene showed that there were some changes in the expression for the four tested genotypes. While, *SbBADH2* gene showed a different behavior than *SbBADH1* gene. However, A2 was the most tolerant genotype, while B8 was the most sensitive one to the salt stress treatment which agreed with the results of the previously recorded salt tolerance-related traits. Finally, these results could be used by breeders to develop new breeding protocols for salt tolerance improvement in sorghum.

Keywords: *Sorghum bicolor*, Salinity, DNA, RNA, ISSR, UPGMA,

PCoA, Polymorphism, ANOVA, LSD, RT-PCR, *SbBADH*.

CONTENTS

	Page
LIST OF TABLES	III
LIST OF FIGURES	IV
LIST OF ABBREVIATIONS	VI
INTRODUCTION	1
REVIEW OF LITERATURE	4
1. <i>Sorghum bicolor</i>	4
2. ISSRs analysis	7
3. Salt stress	9
4. Salt tolerance related traits	11
5. RT-PCR	13
6. BADH	15
MATERIALS AND METHODS	19
1. Plant Material	19
1.1. Cultivating in greenhouse	20
2. Methods	20
2.1. For DNA extraction	20
2.2. For salt tolerance related traits	20
2.3. For RNA extraction	20
2.4. Sterilization	21
2.5. Buffers preparations:	21
2.5.1. ETDA buffer preparation	21
2.5.2. CTAB buffer preparation	21
2.5.3. Loading buffer (TAE 50X) buffer preparation	21
2.6. DNA extraction by CTAB method	22
2.7. Gel electrophoresis for DNA, RNA and PCR product	23
2.8. ISSR-PCR amplification	23
2.9. ISSR Data analysis	25
2.10. Salt tolerance related traits evaluation	25
2.11. RNA extraction	26

2.12. cDNA synthesis	28
2.13. Design of qPCR primers for reference and target genes	28
2.14. Semi qPCR	29
2.15. RT-PCR	30
2.16. Relative quantification	31
RESULTS AND DISCUSSIONS	32
3.1. DNA extraction	32
3.2. Molecular identification using ISSR polymorphism	34
3.3. Genetic distance based on ISSR analysis	40
3.4. Statistical analysis of morphological data	42
3.5. RNA extraction	51
3.6. Normalization of semi qPCR with PP2A and semi qPCRfor <i>SbBADH 1</i> and <i>SbBADH 2</i>	53
3.7. Molecular evaluation using RT-PCR	56
SUMMARY	65
REFERENCES	68
ARABIC SUMMARY	

LIST OF TABLES

Table No.		Page
1	Names and codes of the 20 used (A & B) sorghum genotype.	19
2	Names and sequences of the 10 used ISSR primers.	24
3	Name of genes, gene's symbol, forward and reverse designed primers sequence of semi q-PCR, annealing temperature, accession number and cellular function.	29
4	Measures of spectrophotometer and concentration for DNA of the ten A genotypes.	33
5	Measures of spectrophotometer and concentration for DNA of the ten B genotypes.	34
6	Total no. of alleles, polymorphism percentage, band size, and the highest frequency band of the 20 different sorghum genotypes.	39
7	Mean square of the four genotypes of sorghum for the recorded salt tolerance related traits.	45
8	Mean performance of the four genotypes of sorghum for the recorded salt tolerance related traits.	48
9	Nanodrop's recorded data for the extracted RNA from the most tolerant genotypes (A2 & B5) and the most sensitive genotypes (A9 & B8) at 5 different times (0, 2, 4, 24 and 48 hours) of treatment with saline water.	53

LIST OF FIGURES

Fig. No.		Page
1	Result of DNA extracted from 10 A genotypes.	33
2	Result of DNA extracted from 10 B genotypes.	33
3	Results of amplification based on the use of 10 ISSR primers respectively in the 10 studied sorghum A genotypes from A1 to A10.	37
4	Results of amplification based on the use of 10 ISSR primers respectively in the 10 studied sorghum B genotypes from B1 to B10.	38
5	Dendrogram tree showed genetic distance among the 20 studied sorghum genotypes.	41
6	PCoA analysis showed genetic relationships among the 20 studied sorghum genotypes.	42
7	Averages charts of salt tolerance related traits recorded data.	49
8	Random samples shows the negative effect of salt stress on sorghum from yellow curved leaves.	50
9	Comparison between each genotype one month old controlled and treated with salinity.	51
10	Results of RNA extraction and its integrity. Two bright bands were corresponded to ribosomal 28S rRNA and 18S rRNA.	52
11	Results of Semi q-PCR normalization using reference gene <i>PP2A</i> . Ladder: 1 Kb, 50+ and <i>Hind III</i> .	54
12	Results of Semi q-PCR for <i>SbBADH1</i> gene. Ladder: 1Kb, 50+ and <i>Hind III</i> .	55
13	Results of Semi q-PCR for <i>SbBADH2</i> gene. Ladder: 1Kb, 50+ and <i>Hind III</i> .	55

- | | | |
|-----------|--|----|
| 14 | Molecular evaluation of A2, A9, B5 and B8 genotypes using RT-PCR for <i>SbBADH1</i> gene at 5 different times of salt treatment. | 58 |
| 15 | Molecular evaluation of A2, A9, B5 and B8 genotypes using RT-PCR for <i>SbBADH2</i> gene at 5 different times of salt treatment. | 60 |